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EXPRESSION OF HORMONE-SENSITIVE LIPASE IN THE HUMAN COLON ADENOCARCINOMA CELL LINE HT29

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Hormone-sensitive lipase expression was studied in the human colon adenocarcinoma cell line, HT29. Diacylglycerol lipase and cholesterol oleate activities in HT29 cells were inhibited by known inhibitors of hormone-sensitive lipase (diethyl-*p*-nitrophenyl phosphate, NaF and $HgCl_2$) to the same extent as in human adipocytes. A polyclonal antiserum directed against rat hormone-sensitive lipase inhibited 89 % of HT29 cell lipase activity. HT29 hormone-sensitive lipase was the same size as the adipocyte enzyme as was its mRNA. Complete homology between mRNA sequences in HT29 and adipocyte was demonstrated using ribonuclease protection assay. These data are consistent with the expression of a protein closely related, if not identical, to the enzyme expressed in human adipose tissue. HT29 is the first human cell line where hormone-sensitive lipase expression has been shown.

Hormone-sensitive lipase (HSL; EC 3.1.1.3) has a critical role in the control of energy homeostasis by catalyzing the rate-limiting step in adipose tissue lipolysis, i.e. the hydrolysis of triacylglycerols to diacylglycerols. The subsequent hydrolysis of diacylglycerols to monoacylglycerols is also catalyzed by HSL (1). Free fatty acids released during lipolysis are used by a wide range of energy requiring tissues. Unlike other triacylglycerol lipases, HSL is under

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Abbreviations:
HSL, hormone-sensitive lipase; DNP, diethyl-*p*-nitrophenyl phosphate.

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acute neural and hormonal control, that is activated by various hormones and neurotransmitters through cAMP-dependent phosphorylation of a single serine residue (2).

HSL expression is not restricted to adipose tissue. The protein is present in adrenals, ovaries, testes, placenta, mammary gland, heart and skeletal muscle (3,4). In some of these tissues, HSL may act as a cholesterol esterase since it has the same catalytic activity towards cholesterol esters and triacylglycerols. The human HSL gene has recently been cloned (5). It encodes a protein which shows ≈ 85% homology with the rat enzyme (6). The putative functional domains of HSL are encoded by different exons. In particular, exons 6 and 8 encode for the catalytic site serine and the phosphorylation sites, respectively.

No human cell line has been shown to express HSL. Such a model would be very useful to study the regulation of human HSL gene expression. In this article, we report the expression of HSL in the human colon adenocarcinoma cell line HT29. The data are consistent with the expression of a protein closely related, if not identical, to the enzyme expressed in human adipose tissue.

Material and Methods

Cell lines and tissues. HT29 and Caco2 cell lines were cultured as previously described (7). Human breast adipose tissue was collected immediately after surgery. Isolated fat cells were prepared according to Rodbell's procedure (8). Cells were homogenized in 0.25 M sucrose, 1 mM EDTA, 1 mM dithioerythritol, 10 μg/ml leupeptin and 10 μg/ml antipain and stored at -80°C until analyses. When indicated, HT29 homogenates were centrifuged at 110 000 x g for 60 min at 4°C. The pellet, after resuspension in the homogenization buffer, and the supernatant were stored at -80°C until analysis. Enzymatic activities of human adipocytes were determined on 110 000 x g fat-depleted supernatants since triacylglycerols of the lipid droplet interfere with the assays and 80% of HSL activity in human adipose tissue is found in the supernatant (9).

Enzyme assays. A phospholipid-stabilized emulsion of a dioleyl ether analogue, 1(3)-mono[^{3}H]oleoyl-2-oleylglycerol, and [^{3}H]cholesterol oleate were used to assay the diacylglycerol lipase and the cholesterol esterase activity of HSL (10). Inhibitors (100 μM DNP; 100 mM NaF or 100 μM $HgCl_2$) were added directly to the incubation mixture containing enzyme samples in 20 mM potassium phosphate (pH 7.0), 1 mM EDTA, 1 mM dithioerythritol (omitted when using $HgCl_2$) and 0.2 mg/ml bovine serum albumin. 10-fold diluted antibodies against rat adipose tissue HSL (whole hen plasma) was preincubated for 60 min at 37°C with enzyme samples in the same buffer as above before addition of the substrate and determination of the activity. Protein was determined according to Lowry (11) using bovine serum albumin as a standard.

Partial purification of HSL from HT29 cell. The HT29 homogenate was solubilized with 1% $C_{13}E_{12}$, an alkylpolyoxyethylene ether (Berol 058, Berol Kemi AB, Stenungssund, Sweden) and 10 mM NaCl using sonication with a Branson Sonifier 250 at setting 1-2 (2-3 min in 30 sec pulses cooling with ice). Particulate material was pelleted by centrifugation at 110 000 x g for 10 min at 4°C and discarded. The supernatant was dialyzed overnight at 4°C against 20 mM Tris-acetate, pH 7.5, 20% glycerol, 1 mM dithioerythritol, 0.2% $C_{13}E_{12}$, 2 μg/ml leupeptin. The dialyzed material, corresponding to 180 μg of protein, was purified on a 1 ml Q-Sepharose FF (Pharmacia, Sollentuna, Sweden) column, essentially

as described for purification of rat adipose tissue HSL on Mono Q column (12). The eluate from the column was collected in fractions, which were analyzed for the presence of HSL by measurement of diacylglycerol lipase activity and by Western blotting. Fractions shown to contain HSL were pooled and analyzed for inhibition by antibodies directed against rat HSL.

Western blotting. SDS-PAGE analyses were performed as described (13) with modifications (14), after which proteins were electroblotted onto nitrocellulose membranes. Western blot analyses were carried out using a polyclonal rabbit antibody against rat adipose tissue HSL (3, 15), anti-rabbit IgG conjugated to alkaline phosphatase, and a chromogenic substrate (Protoblot II AP system, Promega, Madison, Wisconsin).

RNA preparation, Northern blot analyses and ribonuclease protection assays. Total RNA was prepared using a single-step guanidinium thiocyanate-phenol-chloroform extraction (16). RNA samples were electrophoresed in a 1% agarose, 2.2 M formaldehyde gel, transferred to nylon and UV-crosslinked to the membrane. The blots were hybridized at 68°C in QuickHybrid (Stratagene, LaJolla, CA) solution with a 1.3 kb human HSL cDNA probe (5) radiolabeled with [^{32}P]dCTP and washed at a final stringency of 15 mM NaCl, 1.5 mM trisodium citrate and 0.1% SDS at 68°C. Ribonuclease protection assay was carried out as described previously (7, 17) using a 453 bp human adipocyte HSL cRNA probe (9).

Results

Enzymatic activity determination on cell homogenates indicated the presence of a diacylglycerol lipase in HT29 (6.11 ± 0.91 nmol fatty acids/min/mg protein, n=8) but not in Caco-2, two human colon adenocarcinoma cell lines. 65% of the activity was found in the 110 000 x g supernatant and 35% in the pellet (n=3). The effect of known inhibitors of HSL were tested on HT29 homogenates and compared to human adipocyte fat-depleted supernatants. Since HSL hydrolyzes triacylglycerols and cholesterol esters, both activities were measured (Table 1). 100 μM DNP, 100 μM $HgCl_2$ and 100 mM NaF almost completely inhibited the diacylglycerol lipase and cholesterol esterase activities of HT29 cells and human adipocytes. The same inhibition profiles were obtained on HT29

TABLE 1. Inhibition of diacylglycerol lipase and cholesterol esterase activities in HT29 cells and human adipocytes. HT29 homogenates and human adipocyte 110 000 x g supernatant were assayed for diacylglycerol lipase and cholesterol esterase activities using 1(3)-mono[^{3}H]oleoyl-2-oleylglycerol and [^{3}H]cholesterol oleate as substrates. Values are mean ± S.E. (n = 3-4).

Inhibitors	Diacylglycerol lipase activity (% inhibition)		Cholesterol esterase activity (% inhibition)	
	HT29	Adipocyte	HT29	Adipocyte
DNP, 100μM	93.6±2.5	91.4±3.0	98.6±0.5	97.3±1.0
NaF, 100mM	67.0±7.4	82.4±6.2	72.4±2.4	72.9±4.6
$HgCl_2$, 100μM	93.4±2.4	91.4±1.0	99.1±0.4	97.8±0.5

TABLE 2. Inhibition of diacylglycerol lipase activity with a polyclonal hen antibody directed against rat HSL. The samples were preincubated with 10 µl preimmune hen serum (control values) or 10 µl antiserum for 60 min at 37°C before addition of 1(3)-mono-oleoyl[^{3}H]-2-oleylglycerol and lipase activity determination. Values are mean ± S.E. (n=5).

	% inhibition
Rat adipose tissue 110 000 x g infranatant	91.5±0.7
Recombinant rat HSL	98.3±0.5
HT29 cell homogenate	74.8±1.7
Partially purified HT29 cell homogenate	89.2±1.4

supernatant and pellet or when cholesterol octanoate was used as a substrate (not shown).

The presence of HSL in HT29 cell homogenate was further supported by the 75% inhibition of diacylglycerol lipase activity obtained when using a polyclonal antiserum directed against rat HSL (Table 2). Because of its low abundance compared to adipose tissue, the lipase activity was partially purified by detergent-solubilization followed by anion exchange chromatography on Q-Sepharose. The partially purified lipase activity was inhibited to 89% by the antiserum. Furthermore, the HT29 HSL protein was identified by Western blotting analysis (Fig. 1). One major protein band and proteolytic fragments cross-reacting with the antiserum were detected. The immunoreactive protein species had the same apparent molecular weight (88 kDa) as human adipose tissue HSL (not shown).

Ribonuclease protection assay was used to detect the presence of HSL mRNA in HT29 cells (Fig. 2). This method was chosen since a few mismatches between the cRNA probe and the template will provoke a degradation of the cRNA-RNA hybrids by the mixture of RNAses. Moreover, this method is more sensitive than Northern blotting (17). A single band was detected in HT29 cells and adipose tissue. No protected band was observed with Caco2 cell total RNA. The size of the mRNA was determined using Northern blot analysis (Fig. 3). A single mRNA species of approximately 3.3 kb was detected in HT29 cells and adipose tissue.

Discussion

In search for a human cell line expressing HSL, diacylglycerol lipase activity was detected in the human colon adenocarcinoma cell line, HT29. Several lines of evidence showed that this activity was due to HSL expression in HT29 cells. The effect of inhibitors was similar in HT29 cells and human adipocytes.

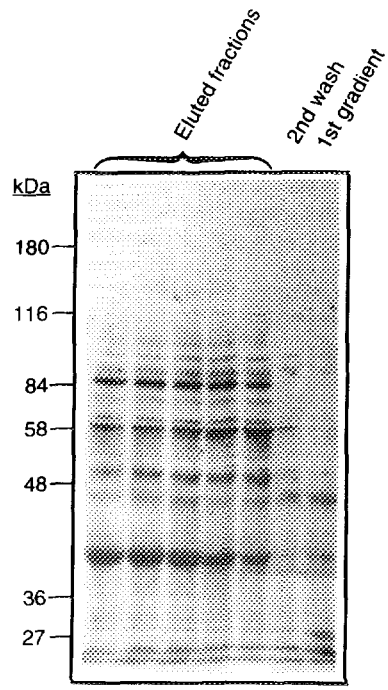

FIG. 1. Identification of HSL from a partially purified HT29 cell homogenate using Western blot analysis. Aliquots of eluted fractions, collected from the Q-sepharose column during the second salt gradient (12) and shown to contain diacylglycerol lipase activity, were subjected to Western blot analysis using a polyclonal rabbit antibody directed against rat adipose tissue HSL. For comparison, aliquots of material from the first gradient and the second wash are shown (12). The mobilities of reference proteins are indicated: α_2-macroglobulin (180 kDa), β-galactosidase (116 kDa), fructose-6-phosphate kinase (84 kDa), pyruvate kinase (58 kDa), fumarase (48.5 kDa), lactic dehydrogenase (36.5 kDa) and triosephosphate isomerase (26.6 kDa).

The same inhibition profile was found when using different types of substrates, i.e. a diacylglycerol analogue, cholesterol oleate (C18 fatty acid) and cholesterol octanoate (C8 fatty acid). HSL is the only known triacylglycerol lipase which shows high activity towards cholesterol esters and releases long- and short-chain fatty acids. The diacylglycerol lipase was found to be mainly cytosolic although a significant fraction was associated with the particulate compartment. This distribution of the activity between the subcellular fractions is similar to the distribution found in 3T3-L1 mouse adipocytes (18). A cytosolic triacylglycerol lipase with an optimum pH around 7 has been described in several tissues (19). However, the involvment of this enzyme in the activity measured in HT29 cells is not likely to be important since this other neutral lipase is not inhibited by DNP and does not show activity towards cholesterol esters. Moreover, the inhibition of the activity by preincubation with a polyclonal antiserum directed against rat HSL was a very strong indication of the presence of HSL in HT29 cells. Western and

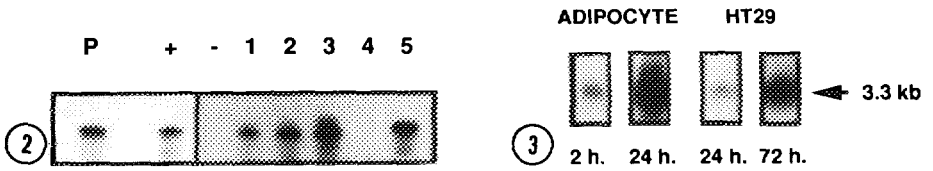

FIG. 2. Detection of HT29 and human adipocyte HSL mRNA using ribonuclease protection assay. Lane P, 1/30th of undigested human HSL cRNA probe used in each assay; lane +, hybridization with synthetic human HSL RNA (exposure time, 6 h). Lane -, hybridization with yeast tRNA; lanes 1 to 3, hybridization with 50, 100 and 200 µg HT29 total RNA; lane 4, hybridization with 100 µg Caco2 total RNA; lane 5, hybridization with 10 µg human adipocyte total RNA (exposure time, 48 h).

FIG. 3. Northern blot analysis of HT29 and human adipocyte total RNAs. 100 µg HT29 total RNA and 25 µg human adipocyte total RNA were electrophoresed, transferred to nylon membrane and hybridized to a 1.3-kb human HSL cDNA probe (5).

Northern blot analyses showed that HT29 HSL was the same size as the human adipocyte enzyme as its mRNA (20). Complete homology between mRNA sequences in HT29 and human adipocyte was demonstrated using ribonuclease protection assay. It is therefore possible to conclude that the proteins in the two tissues are encoded by the same gene and that they are probably identical.

HSL expression in HT29, a human colon cancer cell line, is a somewhat surprising finding. HSL mRNA was not detected in rat intestine (6). Accordingly, we did not detect, using ribonuclease protection assay, HSL mRNAs in normal human jejunal and colonic epithelial cells (not shown) or in the human colon cancer cell line, Caco2. This pattern of expression is reminiscent of the neurotensin receptor distribution in normal and cancer colonic cells (21). HSL and neurotensin receptor expression in HT29 cells could represent an unmasked ectopic expression of proteins due to malignant transformation. A de-repression or reversion to a fetal phenotype has been shown for other proteins such as sucrase isomaltase and dipeptidylpeptidase IV (22). To evaluate the frequency of HSL expression in colon cancers, we should investigate a larger number of cancer cell lines and resected colonic tumors. It is worth pointing out that increased HSL mRNA levels have been reported in adipose tissue of cancer patients, most of them operated for colorectal cancers (23).

HT29 cells contain significant amounts of cholesterol esters and triacylglycerols (24). HSL could participate in the degradation of these substrates either to supply fatty acids to these metabolically very active cells or to release cholesterol. Cholesterol accumulation in plasma membranes is frequently observed during cell malignancy (25). The presence of HSL could confer to HT29 cells an acute control of fatty acids and/or cholesterol release through hormonal stimulation. Receptors coupled positively to adenylyl cyclase, such as vasoactive intestinal peptide receptors, are present in HT29 cells (22) and the stimulation of

α_2-adrenoceptors is associated with an inhibition of cAMP production (26). Therefore, catecholamines and vasoactive intestinal peptide could modulate the lipolytic activity of these cells.

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